STUDIES ON CYTO - MORPHOLOGICAL VARIATIONS INDUCED BY CHEMICAL MUTAGENS IN *Helianthus annuus* L. (COMPOSITAE)

DISSERTATION

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Dedicated
To
My Beloved
Parents

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Dated: 14.10.06

Certificate

This is to certify that the work presented in the M. Phil dissertation entitled **"STUDIES ON CYTO-MORPHOLOGICAL VARIATIONS INDUCED BY CHEMICAL MUTAGENS IN *HELIANTHUS ANNUUS* L."** is the original piece of work carried out by **Ms. BUSHRA ZAMEER** under my supervision and guidance and has not been submitted or published elsewhere for the award of any other degree.

14.10.06

(Dr. Mohd. Yunus Khalil Ansari)

In the
Name of Allah,
The Beneficent, The Merciful,

And he it is who sends down water from the cloud then we bring forth with it buds of all (Plants), then we bring froth from it green (foliage) from which we produce grain piled up (in the ear); and of the palm-tree, of the sheaths of it, come forth clusters (of dates) with in reach, and gardens of grapes and olives and pomegranates, alike and unlike; be-hold the fruit of it when yields the fruit and ripening of it; most surely there are signs in this for a people who believe.

Surah-al-Anam (Verse 100)

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Bushra Zameer
(Bushra Zameer)

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CHAPTER – 1

INTRODUCTION

Mutation is a sudden heritable change in the characteristics of an organism without involving recombination and crossing over. The term mutation was coined by Hugo de Vries (1901) derived from a Latin word "Mutare" (to change). Since then it is used as a collective term for all kinds of hereditary changes. It may be genotypic or phenotypic resulting from germinal alteration in chromosome structure, in individual genes or in somatic parts.

Mutation can be induced or spontaneous. It is the only method by which allelic differences between genes can arise. Brock (1970) studied the induced mutation and found that it is an alternative to the naturally occurring variations as a source of germplasm for plant improvement programmes and as an alternative to hybridization and recombination in plant breeding.

Mutations are induced at varying levels depending upon the nature, dose and the mode of action of mutagen, viz, macro-and-micro mutations. Macromutations cause large disturbance in the normal development and functioning. Therefore, micromutations are important causing the minimum injury and maximum survival.

Mutations could have harmful as well as useful effects. If they are useful they play an important role in evolution. Induced mutations are found to be successful in yield characters, induction of earliness and alteration in grain and seed quality.

Though there are several attempts to induce mutations by chemical agents (Westergaard 1957, Gustafsson 1969), the first definite evidence that the chemical agents can induced mutations was obtained by Auerbach and Robson (1942).

The direction and rate of mutations vary in different genes. Mutations with large effects cause large disturbances in the normal development and functioning, therefore, mutations with small effects are of importance in evolution.

India is a country with great agroproductivity, but still the needs of its great population are to be met with. The oil seed crops are still in the scientifically sophisticated stage of breeding where breeders would be able to produce large quantities of oil at a reasonable rate so as to conserve and improve the health of the human community which will lead to the prosperity of country and world.

The sunflower (*Helianthus annuus* L.) is a member of the family "Asteraceae" (Compositae) which is considered as the highest evolved family of dicots, and includes 20,000 species. Seiler (1992) found that the genus *Helianthus* is composed of 49 species and 19 subspecies,

with 12 annual and 37 perennial species. The genus *Helianthus* is named from Greek 'Helios' meaning sun and 'Anthos'-meaning flower. The name *Helianthus annuus* L. was given by Linnaeus because the only sunflower known to him lived only annually i.e. for one season.

Sunflower probably originated in South West united states – Mexico area (Vranceanu 1974 ; Heiser 1976). However, as a cultivated crop it was reintroduced to North America from Europe in late 19th century, although sunflower was introduced to Europe in 16th century. The first published description appeared in herbal literature by Rambert and Dodoens in (1568). The first commercial production of oil occurred in 1830-40. The crop has been steadily grown in importance from 1970's and today the sunflower is one of the world's important oil seed. In 1979-80, it was estimated as the record global oil production (Mielke 1980).

The seeds contains a fatty oil (37-48%) which is rich in poly unsaturated acids (linoleic 70%; oleic 20%). The oil also contain to copherols (α type, 92% of the total) and phytosterols (β - sitosterol, 154 µg / 100 gms). The flowers contain triterpenoid soponins, heliantho sides 1, 2 & 3. (wealth of India P : 248-249) 2002.

The seeds of sunflower are diuretic and expectorant. They have been used in bronchial, laryngeal and pulmonary infections, coughs and cold etc. Leaves are reported to be employed in the treatment of

malarial fever in Caucasus. *Helianthus annuus* L. is the source of sunflower oil which is reaching the peaks of popularity with growing health awareness because it is proved to be unsaturated cooking medium and it is cholestrol-free. It minimizes to heart attacks and heart failure. Realising the importance of the sunflower oil, attempts were made to carry out mutation breeding work on *Helianthus annuus* L. The chemical mutagens maleic hydrazide and 6-Benzyl amino purine have been employed in different concentrations.

The assessment of mutagenic effects of these chemical mutagens has been done on different parameters, such as seed germination, seedling growth, survival rate, mutation frequency etc. by observing morphological variations at seedling and mature stages, habit of the plant, nature of flowering, branches yield etc. cytological studies in control as well as treated plants have also been done.

GENERAL DESCRIPTION OF *HELIANTHUS ANNUUS* L. :

Habit : Annual cultivated herb.

Root : Taproot, branched, fibrous.

Stem : Erect, herbaceous above, woody below, cylindrical, glabrous, branched, fistular with swollen nodes.

Leaf : Cauline and ramal, petiolate, opposite extipulate, simple, ovate, serrate, acute, unicostate reticulate

venation; frequently leaves are radical and crowded in rosettes.

Inflorescence : A head or capitulum (heterogamous head) consisting of two kinds of flowers, the disc florets, the central ones, bisexual and tubular; the ray-florets the marginal, ones; pistillate or neuter and ligulate; small scaly bracts called the palae are present between the florets; capitulum paleaceous.

Ray-florets : Bracteate, ebracteolate, sessile, incomplete, irregular, zygomorphic, ligulate, pistillate or neuter, epigynous, yellow; each floret arises in the axil of the bracteole.

Calyx : Represented by 2-3 or more small scales persistent (pappus).

Corolla : Petals 2-3, yellow, gamopetalous, a short basal tube and a large flat strap – shaped limb, with 2-3 teeth, ligulate, valvate aestivation.

Androecium : Absent.

Gynoecium : Absent.

Disc-floret : Bracteate, ebracteolate, sessile, regular, tubular, actinomorphic, bisexual, epigynous, pentamerous, complete.

Calyx : Reduced, modified into 2-3 or more scales (pappus), Persistent.

Corolla : Petals 5, gamopetalous, epipetalous, tubular, toothed, valvate aestivation.

Androecium : Stamens 5, epipetalous; filaments free, short, alternating with the petals, syngenesious i.e. anthers united, filaments free; anthers basifixed, introrse.

Gynoecium : Bicarpellary, syncarpous; ovary inferior, unilocular, basal placentation; style long, terminal; stigma bifid and hairy.

Fruit : Cypsela.

Seed : Non-endospermic.

CLASSIFICATION

Kingdom : Plantae

Class : Dicotyledonae

Sub-Class : Gamopetalae

Series : Inferae

Order : Asterales

Family : Asteraceae

Genus : *Helianthus*

Species : *annuus*

Review of Literature

CHAPTER - 2

REVIEW OF LITERATURE

Induction of mutations through physical and chemical mutagens is considered as an alternative source to naturally occurring variability for crop improvement programmes. Conventional methods of breeding have been extensively used for crop improvement, but the lack of genetic variability limits the scope of improvement through selection or hybridization. Induced mutagenesis plays a very important role in enhancing new genetic variability. Induced mutations are random, polydirectional and quantitative in nature and bring about heritable changes in polygenic system (Ignacimuthu and Babu, 1993).

The idea of inducing mutations and utilizing them for improving cultivated plants is about eighty years old. For the first time when Muller (1927) succeeded in inducing mutations in a fruitfly *Drosophila melanogaster* by X-rays, it marked the beginning of a new era in genetics and induced mutations and became the focus of genetic studies. Pioneer studies on induced mutations in plants were undertaken by Stadler (1928). Scientists working on different aspects of mutagenesis have since then been able to accomplish a significant breakthrough in understanding the mechanism of mutagenesis and also its applied value for the benefit of mankind.

The possibility of induction of mutations by the use of chemical mutagens started appearing within a decade after discovery of the phenomenon. However, the first elaborate report was presented by Auerbach and Robson (1942) who showed that mustard gas could induce mutations as well as chromosomal changes in Drosophila. Mutagens have remarkable possibilities of improving plants with regard to their qualitative and quantitative characters and where appropriate selection method has been applied.

Our knowledge in the fundamental aspects of mutational processes and the mechanism of action of various physical and chemical mutagens have been fairly widened with the reports of Blixt and Gottschalk (1975) and Gottschalk and Wolf (1983) and Sharma (1985).

Improvement in yield (Brock, 1965; Gregory, 1968), adaptability (Gustafsson, 1965), maturity time (Brock, 1970), disease resistance (Yamasaki and Kawai, 1968) and numerous other traits (sigurbjornson and Micke, 1969) have also been reported by mutagenic treatments. The generation of genetic variability through induced mutagenesis provides a base for stregthening plant improvement programmes.

MALEIC HYDRAZIDE (MH) : ($C_4H_4N_2O_2$)

Maleic hydrazide (MH) has not been studied extensively for its ability to induce point mutation either in procaryotes or eucaryotes. However, chromosome breaking effects of MH in plants were first described by Darlington and Mc Leish in (1951). It is known to induce localized chromosome breakages and mitotic suppression in *Vicia faba* root meristem. It also causes inhibition of spindle formation and chromosome breakage during mitosis in root tips of onion and barley (Kaul and Chaudhary 1975).

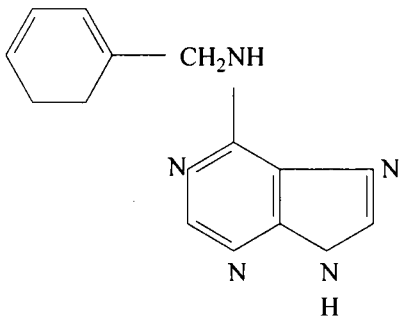
Cytological effects of MH treated plants have been studied by many workers and it has been reported that MH induces chromosomal damage and also different types of chromosomal aberrations (Meschini *et al.*, 1988, De-Marco *et al.,* 1992). Misra (1995) reported fifty four induced mutants of black gram (*Vigna mungo*) line T-9, which were developed by single and combined treatment with chemical mutagens ethyl methane sulphonate, sodium azide, N-methyl-N-nitroso-guanidine and maleic hydrazide. Genetic variability has been reported to increase in quantitative characters as a result of MH treatment in green gram (Khan *et al.,* 1998).

Maleic hydrazide (MH) has been recognized as plant growth inhibitor. Structurally it is an isomer of uracil (a pyrimidine compound of RNA). The mode of action of MH is possibly through its

interference with synthesis of uracil or becoming incorporated into RNA molecule, replacing the uracil or its reaction with sulphydryl groups of nucleic acids. The final result in any case is presumably a weakness in the structure of chromosome leading to chromosome breakage (Grant and Harney 1960, Heslot, 1977). Cortes *et al.* (1987) are of the opinion that the cytogenetic action of MH resembles to the bi-functional alkylating agents in many respects. According to Cortes *et al.* (1987) also, its action includes the reaction with phosphate group of DNA or purine base especially the guanine of DNA.

6-BENZYLAMINO PURINE (6-BAP) : ($C_{12}H_{11}N_5$)

6-Benzylamino purine (6-BAP) is a cytokinin. Its structure is as follow :



6-BAP has generally been used as a hormone, very less work has been done on it as a mutagen, although amino purine group of this compound takes part in mutation. Amino purine group can pair with thymine (T) by two hydrogen bonds and with cytosine (C) by a single bond. The pairing involving two bonds is more common, since in the

other case nitrogen (N) in amino-purine and nitrogen (N) in cytosine (C) repel each other and cause their separation. Incorporation of AP at the place of guanine (G) to give AP-C base pair will cause mutation in subsequent generations. Similarly a mistake in replication after incorporation of AP leading to the formation of AP-T base pair may lead to mutation. Aminopurine thus induces transitions in both directions.

GENE ACTION :

Anisimova (1991) found Helianthenin (sunflower main storage protein, 115 globulin) can serve as a marker character in analysis of pure line varieties and hybrid varieties.

Jain (1992) studied the Nuclear Male Sterility (NMS) mutants derived from inbred lines 'HA-89' and two lines 'BAIIA3 and 'P21' for their mode of inheritance, allelic relationship and programic characters.

HYBRIDIZATION :

Hybrids of inbred lines of sunflower have given the biggest increase in yield and oil contents and are in general commercial use. Named hybrids of known performance are now available in many countries from reputable seed companies. The introduction of hybrid sunflower in late 1970's has radically improved the profitability and

therefore long term prospects for the crop. Hybrid sunflower has important advantages over open-pollinated varieties. It produces very high seed yield, increasing seed oil content, flowers over a short period and has a short, sturdy stem with a single head. All these characteristics increase the amount of mechanization possible and add to its efficiency. Many hybrids are also drought resistant or resistant to some insect pests or diseases.

Vegetative hybridization has been reported from Russia, with grafts of sunflower on *Xanthium strumarium*. Kinman (1970) produced hybrid sunflowers and cultivars with short stems.

Cokerell (1979) studied hybrid sunflower Autosyndesis and structural hybridity in F_1 hybrids, *Helianthus tuberosus* L. crossed with *Helianthus annuus* L. and their sequences. Bohorava and Georieva (1987) reported hybridization between *Helianthus annuus* L. (2n=34) and *Helianthus hirsutus* (2n=68) and concluded that pairing had occurred mainly between parently homologous chromosomes of both species.

Kristov (1988) found that in the course of hybridization at conditions existing in the field crosses between *Helianthus agrophyllus* and the cultivated sunflower (*H. annuus* L.) are relatively easy and interspecific incompatibility is manifested at stages of embryonal development. Jordanka (1988) analysed F_1 hybrids between

Helianthus annuus and *Helianthus decapetalus*. The study on the effect of back-crossing on Cytological and phenotypical stabilization substantiate the conclusion that both are used in sunflower hybridization programmes. Gubbels and Dedio (1988) detected that plant height was reduced and echene yield was increased for early hybrid of the sunflower. Deibert (1989) observed that early maturing sunflower hybrids are better adapted to dry land areas of cool northern states, because seed yield and quality irrespective of tillage or weed control methods were equal to or better than those for late maturing hybrids.

Georgieva and Lokova *et al.* (1978) found that *H. annuus* hybridization with *H. tuberosus*, *H. rigidus* and *H. resinosus*, yields hexaploid spe dies. The F_1 hybrids were characterized by their high sterility. Fertility was raised thought not completely only after 3 or 4 back crosses. Hybrids between *H. annuus* and *H. rigidus* were produced with difficulty. The pre-existing high incompatibility between these two species were partially over come when *H. rigidus* was used as female. It was also found that *H. resinosus* was more closely related to the cultivated sunflower than other hexaploid species (*H. tuberosus*, *H. rigidus*) and that the development of all hexaploid species had not been attained in the same way.

STERILITY AND INCOMPATIBILITY :

Throughout the entire plant kingdom species annual flowering plants tend to be easily, if not obligatory, self-fertilized. The term sterility generally includes all those cases where there is failure of production of viable offsprings-fruits, seeds or even any of the products expected by man.

Leclerq (1969) found Cytoplasmic male sterility in crosses between *Helianthus petiolaris* and *Helianthus annuus* combined with fertility restorer genes. Kinman (1970) produced hybrid sunflowers with short stem suitable for mechanical production. Ivanov (1975) studied the compatibility display in crossing selfed sunflower lines. Vranceanu and Stoenescu (1978) studied the genes for pollen sterility restoration in sunflower and emphasized the influence of different genetic and environmental factors on pollen self-compatibility in sunflower.

Male and female sterility can also be chemically induced (Tores *et al.*, 1979). Whelan (1981) observed cytoplasmic male sterility in the hybrid of a cross between *Helianthus giganteus* and *H. annuus*. George and Shein (1980) pointed out the effect of stigmatic manipulation on pollination and seed set in sunflowers. Leroy *et al.* (1986) are of the opinion that mitochondrial DNA is related with male sterility and fertility in sunflower. Crouzillat *et al.* (1987) described the molecular

analysis of mitochondrial genome of *Helianthus annuus* in relation to cytoplasmic male sterility and phylogeny and concluded that the mitochondrial plasmid P_1 was cloned, translated and hybridized with mitochondrial DNA from different lines of fertile male. Swamy and Griraj (1989) evaluated the sunflower populations for self compatibility over seasons.

CYTOLOGY :

Majority of *Helianthus annuus* L., the common sunflower, has the somatic number (2n=34). It also occurs in polyploid conditions having 2n=68 (4x), 2n=102 (6x). Exceptionally 2n=14, 28 or 32 are also found (Prokopenko, 1975).

Tanaka (1967) pointed out a comparative karyotype analysis such as breakage, bridges and laggards in *Haplopappus gracilis* (2n=4) and *H. ravenii* (2n=8).

Gupta (1969) and Gupta *et al.* (1972) carried out the cytological investigations in some Indian compositae and Singhvi (1974) studied the karyomorphology in some desert compositae. A comparative study of cytogenetic effects of two culture media on suspension of *Haplopappus gracilis* was done by Singh (1976). He observed that the frequency of abnormal anaphase configuration was higher. Single and paired bridges and cells with paired fragments have been observed in *H. annuus* after post gamma irradiation (Shizova, 1976).

Werry *et al.* (1977) concluded that the relative position of interphase chromosomes was reflected by the relative position of metaphase chromosomes in *Haplopappus gracilis*. In *Helianthus salicifolius* the chromosome morphology suggests the diploid genome of the species resulting in failure of interspecific crosses (Georgieva and La Kova, 1978). Al-Allaf and God ward (1979 a,b) analysed the karyotype of four varieties of *Helianthus annuus* (2n=34) and observed that metaphase from each variety revealed no varietal difference so far as chromosome morphology and the position of the centromeres was concerned. In a karyotype study the chromosomal arrangement pattern and behaviour in normal and caffeine treated binucleate meristematic cells of the root tips of *Haplopappus gracilis* (2n=4) was in most of the cases same (Tanaka, 1967). Mathew and Mathew (1982) also carried out the detailed karyomorphological studies of eight species of Vernonia and nine species of Blumea of the family compositae.

The occurrence of chromatin bridges and laggards during meiosis in 2,4-D treated progeny (Siddiqui *et al.*, 1980) has been reported in *Helianthus annuus*. Yoshihiko (1981) observed a 'J' shaped chromosomes in *Haplopappus gracilis* and the reason of this transposition of centromere according to him was presumed to be an actuation of the supposed centromere in the subterminal position. Ashmore and Shapcot (1989) obtained polyploids by callus and

suspension cell cultures. In polyploids, the chromosome loss and rearrangement occurred to give rise to aneuploids and chromosome bridges at anaphase in *Haplopappus gracilis.*

By colchicine treatment the karyotypic changes such as breakage, polyploids and haploids in *H. laciniatus* (x=17) (Celal, 1979) have been produced.

At present an effort has been made to carry out mutational work in compositae, particularly *Helianthus annuus* L. variety 'MSFH-8'. Considering its importance the plant needs more attention in the fields of breeding and mutagenesis.

Materials and Methods

CHAPTER - 3

MATERIALS AND METHODS

In the present work the effect of aqueous solutions of chemical mutagens, maleic hydrazide and 6-Benzyl aminopurine on cytomorphological characters on *Helianthus annuus* L. variety 'MSFH-8' has been studied.

The certified healthy seeds of sunflower variety 'MSFH-8' was obtained from Maco-seed company Maharashtra, Jaalana.

EXPERIMENT PROCEDURE:

PREPARATION OF STOCK SOLUTION:

The 2.0% stock solution of maleic hydrazide (MH) was prepared in distilled water and then diluted to 0.05%, 0.1%, 0.2%, 0.4%, 0.6%, 0.8%, 1.0%, 1.5% and 2.0 % concentrations. The 0.2% stock solution of 6-Benzyl aminopurine (6-BAP) 0.02% and the diluted to 0.005%, 0.01%, 0.02%, 0.04%, 0.06%, 0.08%, 0.1%, 0.15% and 0.2% respectively.

The seeds were presoaked in distilled water for 6 hours at room temperature, therefore treated in each concentration of mutagens for 24 hours.

The treated seeds after 24 hours were throughly washed in running water in order to remove the mutagens from seeds surface. Three replicates of 100 seeds each were sown for each treatment. The morphological observations were recorded from treated as well as control populations.

OBSERVATIONS IN M_1 GENERATION:

A detail study of the effects of MH and 6-BAP treatments was done using the following parameters.

SEED GERMINATION:

Germination data were recorded every alternate days, upto 30 days after sowing, till the maximum germination was attained.

The germination percentage was calculated by the following formula.

$$\text{Germination percentage} = \frac{\text{No. of seeds germinated}}{\text{No. of seed sown}} \times 100$$

The delaying effect of mutagens on germination was recorded on the basis of extra days taken for germination in the treated populations as compared to control.

PERCENTAGE INHIBITION IN GERMINATION:

Percentage inhibition in germination or seedling injury was calculated by using following formula:

$$\text{Germination inhibition} = \frac{\text{Number of seeds germinated in control} - \text{Number of seeds germinated in treated population}}{\text{Number of seeds germinated in control}} \times 100$$

SEEDLING AND PLANT MORPHOLOGY:

The parameters of morphological variations were the size and shape of cotyledonary and vegetative leaves at seedling stage and branching and leaf morphology at mature stage in control and treated populations.

FREQUENCY OF MORPHOLOGICAL VARIATIONS :

Following method was adopted to calculate the frequency of these variations.

$$\text{Variation frequency (\%)} = \frac{\text{No. of varied seedlings}}{\text{Total No. of germinated seedlings}} \times 100$$

PLANT HEIGHT:

The height of plants were recorded from the point above the ground to the tip of main axis, at maturity stage.

CYTOLOGICAL STUDIES

(i) FIXATION OF FLOWER BUDS:

For the study of meiosis the young flower buds of proper size were fixed, between 8:00 to 10.30 a.m. in carnoy's fluid (6 parts absolute alcohol : 3 parts chloroform : 1 part glacial acetic acid) for 40-45 minutes or until complete dissolution of chlorophyll. Buds were then transferred to propionic acid (saturated with ferric acetate) for 24 hrs, washed with 70% alcohol and stored in it.

(ii) PREPARATION OF SLIDES:

Anthers were squashed in 0.5% propionocarmine stain (swaminathan *et al.*, 1954). The slides were dehydrated in Normal Butyl alcohol series (Bhaduri and Ghosh, 1954), mounted in canada balsam and dried in incubator at 45^{0}C for 3-5 days. Cytological observations and microphotographs were taken from temporary as well as permanent slides.

CHIASMATA FREQUENCY:

The number of chiasmata per cell and per bivalent was estimated in treated as well as control plants by scoring pollen mother cells (PMCs), at random, at diakinesis & metaphase Ist stage.

MEIOTIC ABNORMALITIES:

Randomly selected PMCs were analysed at diakinesis to telophase stages. The parameter taken into account were chiasma frequency, multivalents, stickiness of chromosomes, unequal divisions, bridges, laggards breakage, precocious separation and micronuclei etc.

POLLEN FERTILITY:

Mature anthers of the randomly selected control and treated plants were squashed in 1% acetocarmine. Fully stained, full size pollen grains with smooth and regular outline were counted as fertile, while unstained, empty, shrunken and deshaped pollen grains were counted as sterile. The percentage of pollen fertility in each concentration was calculated by using the formula:

$$\text{Pollen fertility (\%)} = \frac{\text{Number of fertile pollen grains}}{\text{Total number of pollen grains}} \times 100$$

STATISTICAL ANALYSIS

The data recorded on different characters relating to each of the different treatments have been subjected to statistical analysis with a view to find the individual and comparative effects of different mutagens.

MEAN ($\bar{x}$):

The mean was computed by taking the sum of a number of observations and dividing it by the total number of observations recorded. Therefore,

$$\bar{x} = (x_1 + x_2 \text{----------} x_n)/N$$

or $$\bar{x} = \frac{\Sigma X}{N}$$

where x_1, x_2---------- x_n = observations.

N = Total no. of observations recorded.

STANDARD DEVIATION (S.D., α):

Standard deviation is a positive square root of the average of sum of squares of deviations of all observation from their means. It is calculated by the following formula.

$$\text{S.D. or } \sigma = \sqrt{(x_1 - \bar{X})^2 + (x_2 - \bar{X})^2 \text{----------} + (x_n - \bar{X})^2 / N}$$

$$\text{or} = \sqrt{\frac{(x-\bar{X})^2}{N}}$$

$\bar{x}$ = mean of observations involved

x_1----x_n = individual

N = No. of observations

Coefficient of variations (CV):

It measures the relative magnitude of variations present in observations relative to the magnitude of their arithmetic mean. It is defined as the "Rate of standard deviation to arithmetic mean expressed as percentage."

$$C.V. = \frac{S.D.}{x} \times 100$$

Standard Error (S.E.):

Standard error is calculated by dividing the value of S.D. by square root of number of observations:

$$S.E. = \frac{S.D.}{\sqrt{N}}$$

where, S.D. = Standard deviation

N = Number of observations

Observations

CHAPTER – 4

OBSERVATION

The effect of maleic hydrazide and 6-benzylaminopurine on seed germination, seedling growth, morphological characters, meiotic behaviour and frequency of variations in M_1 generation have been studied. For the identification of mutations in M_2, the plants raised from seeds selected from selfed variants of M_1 were compared with the control plants throughout their development and on the basis of M_2 characters and chromosomal studies. To assess the comparative effects of different mutagenic agents the frequency of morphological mutations and meiotic chromosomal abnormalities were analysed statistically.

OBSERVATION IN M_1 GENERATION:

SEED GERMINATION:

Seed germination counts were made on 5th, 10th, 15th, 20th, 25th and 30th days after sowing in control as well as treated seeds. The maximum germination was observed to be 55.33 % in control, while in treated populations it decreased from 54.66 to 42.66 % in 0.05 to 2.0 % Maleic Hydrazide (MH) and 50.66 to 41.33 % in 0.005 to 0.2 % 6-Benzyl Aminopurine (6-BAP) respectively on 30th day (Tables 1 & 3). The germination reduction as a whole was highly affected in both mutagens.

The range of germination injury was 1.20 to 22.89 % in 0.05 to 2.0 % maleic hydrazide (MH) and 1.29 to 19.48 % in 0.005 to 0.2 % 6-BAP (Tables-1&3). The injury was directly proportional to the increasing concentrations of both mutagens. Moreover the injury was comparatively more in maleic hydrazide than in 6-BAP (Tables - 1 & 3).

MORPHOLOGICAL VARIATIONS

The frequency of morphological variations was found to increase with increasing concentrations of MH and 6-BAP. It increased from 3.00 to 18 .00% in 0.05 to 2.0 % MH and 15 to 19 % in 0.005 to 0.2 % 6-BAP respectively. The variations were generally more in 6-BAP than in MH treatments.

THE MORPHOLOGICAL VARIATIONS IN MH TREATMENTS:

The control seedlings of *Helianthus annuus* L. were erect, bearing simple ovate, serrate and acute leaves and frequently crowned in rosettes whereas, the treated seedlings showed thick, leathery, oblong acute/slightly acuminate leaves in 0.05 % MH (Fig. 2-a) and in 0.1 % MH the seedlings had oval, oblong, broader, acute as well as obtuse leaves (Fig. 2-b).

In 0.2 % MH the cotyledonary leaves were oval or rectangular and vegetative leaves diverted to one side. The leaves exhibited oblong

with obtuse apices, condensed nodes, rough surface and dark green colour. Erect plant showing stunted growth (Fig. 3-b) while in the conc. of 0.4 % MH the seedling showed whorl like arrangement, condensed at the apex, wavy and dissected margin, obovate as well as rhomboid, hairy surface as well as entire margin (Fig. 3-C).

In 0.6 % MH the plant showed slow growth reduced height, oval as well as oblong leaves (Fig. 4-a).

In 0.8 % maleic hydrazide cordate as well as oval oblong leaves with acute or obtuse apices were more common (Fig. 4-b).

In 1.0 % MH the growth of plant was stunted bearing smaller deshaped and rudimentary leaves (Fig. 4-C), whereas other plants showed more slower growth with smaller oval and ovate leaves (Fig. 4-d). In 1.5 % MH the plant growth was more stunted, but the leaves were oblong (Fig. 5-a). Another plant showed broader leaves, deep notching, forming two overlapping lobes (emerginate). All leaves were different regarding shape, size and apices. i.e. cordate/ ovate shapes and acute/ovate apices (Fig. 5-b).

In 2.0 % MH the stem was thick but semi erect, cotyledonary leaves were normal but unequal. All vegetative leaves were rudimentary, condensed at the apex and the plant showed, stunted growth (smallest height), leaves normal but smaller in size (Figs. 5-c,d).

THE MORPHOLOGICAL VARIATIONS IN 6-BAP TREATMENTS:

In 6-BAP treatments the chlorophyll deficiency was fragment. At seedling stage some leaves were round (orbicular) with notching. In other seedlings the leaves were generally smaller in size and ovate (Fig. 6-a). But in 0.01 % 6-BAP the cotyledonary leaves showed normal as well as reduced in size of leaves, ovate or oblong shapes, some leaves highly deshaped (Fig. 6-b). In 0.02 % 6-BAP rudimentary seedlings were more common along with normal seedlings. Moreover, affected seedlings showed reduced percentage of germination.

In 0.04 % 6-BAP the affected seedlings showed ovate, oblong leaves. Moreover, some deshaped and chlorophyll deficient leaves were also recorded (Fig. 7-a, b).

In 0.06 % conc. cordate, deltoid, oblong, chlorophyll deficient and notched leaves were frequent in 6-BAP (Fig. 8-a), while in 0.08 % 6-BAP leaves were cordate, ovate, oblong, rhomboid, slightly notched, chlorophyll deficient along with deshaped cotyledonary leaves (Fig. 8-b).

In 0.1 % 6-BAP the leaves were oval, broader, obtuse as well as acute, While in 0.15 % 6-BAP seedlings showed general variations in shape and size of leaves such as notching, cordate, oblong, ovate etc. some leaves show chlorophyll deficiency (Fig. 9-a & b).

HEIGHT OF THE PLANTS:

The average height of plants at days old seedling stage in control was 17.18 cms, but in treated populations it decreased from 16.14 cms to 10.95 cms in 0.05 to 2.0 % MH. In 6-BAP it decreased from 14.44 cm to 10.61 cms in 0.005 to 0.2 % concentrations (Tables-1 & 3). At mature stage the maximum average height of plants in control was 77.96 cms, while in treated populations it decreased from 75.17 to 67.91 cms in 0.05 to 2.0 % maleic hydrazide and 72.22 to 69.02 in 6-BAP respectively (Tables -2 & 4).

HEIGHT INJURY:

Height injury at seedling stage was observed to be 0.00 % (nil) in control, but in treated populations it increased from 6.05 to 36.26 % in 0.05 to 2.0 % MH and from6.55 to 31.10 in 0.005 to 0.2 % concentrations respectively. (Tables -1 & 3).

Height injury at mature stage in control was 0.00 % (nil) while in treated populations it increased from 3.35 to 12.12 % in 0.05 to 2.0 % MH and from 1.08 to 8.11 % in 0.005 to 0.2 % 6-BAP (Tables -2 & 4).

AVERAGE NUMBER OF BRANCHES:

The average number of branches 0.85 per plant was maximum in control. It decreased from 0.82 to 0.57 per plant in 0.05 to 2.0 %

maleic hydrazide, while in 6-BAP the decrease was from 0.67 to 0.50 per plant in 0.005 to 0.2 % conc. respectively (Tables -2 & 4).

DAYS TO MATURITY:

Days taken by the plants to maturity was maximum 112 days in control and lower doses of both mutagens, but decreased from 102 to 89 days in 0.2 to 2.0 % MH and 102 to 77 days in 0.005 to 0.2 % of 6-BAP respectively (Tables -2 & 4). This is an important achievement provided that it expresses in further generations also.

YIELD:

The yield of control and treated populations was determined on the basis of weight of seeds per plant which was different in all the concentrations of treated populations as compared to control. It generally decreased in higher concentrations of mutagens.

NUMBER OF SEEDS PER PLANT:

Number of seeds per plant was 975 in control, while in treated populations it decreased from 812 to 542 seeds per plant in 0.05 to 2.0 % maleic hydrazide and from 910 to 650 seeds per plant in 0.005 to 0.2 % 6-benzyl amino purine.

WEIGHT OF 100 SEEDS (GMS):

The weight of 100 seeds was 5.75 gms in control, whereas at decreased from 5.67 to 2.69 gms in 0.05 to 2.0 % MH and from 5.68

to 2.17 gms in 0.005 to 0.2 % 6-BAP concentrations. Although the weight of seeds followed a decreasing trend but it did not follow a linear decreasing trend, rather the size of seeds was independent of the increasing doses particularly in MH.

POLLEN FERTILITY:

The average pollen fertility was 98.5 % in control, whereas it decreased from 94.57 to 80.64 in 0.05 to 2.0 % maleic hydrazide and 98.17 to 81.89 in 0.005 to 0.2 % 6-Benzylamino purine. Pollen fertility showed the same linear decreasing trend as observed in other characters. The reduction in fertility was more in maleic hydrazide followed by 6-benzyl amino purine.

MEIOTIC STUDIES IN M_1 GENERATION :

Meiotic studies are important phenomena to estimate the potency of mutagens on the structural and numerical rearrangements. It can be achieved through mutagenesis to create new recombinations, which are rarely obtained spontaneously or by conventional methods.

Helianthus annuus L. has seventeen bivalents (2n=34). The investigation, following mutagenic treatments was confined mainly to the structural changes in chromosomes and their relationship with phenotypic/genotypic variations. The parameters of meiotic studies were the chiasmata frequency, multivalents, laggards, bridges, stickiness, micronuclei and various other chromosomal aberrations.

(I) ABNORMALITIES AT PROPHASE I :

(i) CHIASMATA FREQUENCY PER CELL :

Chiasmata frequency being a genetically controlled character, did not vary unless the plants are subjected to mutagenic treatments. In control plants the chiasmata frequency was 23.55 per cell but decreased in treated populations from 22.4 to 19.29 per cell in 0.05-2.0% MH and from 21.3 to 19.85 per cell in 0.05-0.2% 6-BAP concentrations, respectively (Tables-5 & 6).

(ii) CHIASMATA FREQUENCY PER BIVALENT :

The chiasmata frequency in control was 1.38 per bivalent, at prophase I (diakinesis), but reduced gradually from 1.31 to 1.12 per bivalent in 0.05-2.0% MH and from 1.24 to 1.16 per bivalent in 0.005-0.2% 6-BAP concentrations, respectively (Tables-5 & 6).

(iii) MULTIVALENTS :

Multivalents were not present in control, 0.6% and 1.0% MH concentrations, however their frequency in treated populations ranged between 0.1 and 0.2 per cell in 0.05-2.0% MH and in 0.005-0.2% 6-BAP concentrations, respectively (Tables-5 & 6). Their absence in 0.6

and 1.0% MH showed that they did not necessarily occur in all doses or might have been present but could not be traced.

(iv) UNIVALENTS :

The univalents were 3.9 per cell in control. Their frequencies increased insignificantly from 5.7 to 5.9 per cell in 0.05-2.0% MH, but significantly from 6.35 to 7.7 per cell in 0.005-0.2% 6-BAP respectively (Tables-5 & 6).

(v) STICKINESS :

Stickiness in control was 0.05 per cell, but increased from 0.1 to 0.2 per cell in 0.05-2.0% MH and from 0.1 to 0.15 per cell in 0.005-0.2% 6-BAP concentrations respectively (Tables-5 & 6).

(II) ABNORMALITIES AT METAPHASE I :

(i) CHIASMATA FREQUENCY PER CELL :

Chiasmata frequency was 24.90 per cell in control at metaphase I, but reduced gradually from 21.15 to 19.30 per cell in 0.05-2.0% MH and from 21.65 to 17.40 per cell in 0.005-0.2% 6-BAP concentrations, respectively (Tables-5 & 6).

In 6-BAP treatments the decrease in chiasmata frequency was not linear, because in 0.08 and 0.01% concentrations, it showed increasing trend (Tables-5).

(iii) CHIASMATA FREQUENCY PER BIVALENT :

The chiasmata frequency was 1.46 per bivalent in control but in treated populations it decreased from 1.24 to 1.13 per bivalent in 0.05-2.0% MH and from 1.27 to 1.01 per bivalent in 0.005-0.2% 6-BAP concentrations, respectively (Tables-5 & 6). These followed the trend similar to per cell frequencies.

(iii) MULTIVALENTS :

Multivalents were not present in control at metaphase I Although, their frequencies in treated populations increased in higher doses, ranging between 0.1 and 0.3 per cell, but did not follow a linear trend in MH, whereas increased upto 0.5 per cell in 6-BAP (Tables-5 & 6). Moreover, the multivalents could not be observed in 0.005% and 0.01%.

(iv) UNIVALENTS :

Univalents were 5.00 per cell in control. Their frequencies increased from 5.55 to 7.40 per cell in 0.06% conc., but decreased in

still higher concentrations due probably to the increasing frequency of multivalents (Table-5&6).

(v) STICKINESS :

Stickiness could not be recorded in control. Its frequency increased from 0.1 to 0.2 per cell in 0.05-2.0% MH and from 0.1 to 0.15 per cell in 0.005-0.2% 6-BAP concentrations respectively (Tables-5 & 6).

(vi) PRECOCIOUS SEPARATION :

Precocious separation of chromosomes could not be observed in control. Their frequency increased from 0.1 to 0.45 per in 0.05-2.0% MH and from 0.1 to 0.2 per cell in 0.005-0.2% 6-BAP concentrations respectively (Tables-5&6).

(III) ABNORMALITIES AT ANAPHASE I :

The studies on the abnormalities at anaphase I were mainly concerned with bridges, laggards, unsynchronized movement, unequal separation and stickiness of chromosomes.

(i) BRIDGES :

Bridges could not be recorded in control, but increased from 0.05 to 0.15 per cell in 0.05-2.0% MH and in 0.01-0.2% 6-BAP concentrations respectively (Tables- 5 & 6).

(ii) LAGGARDS :

Their frequencies in treated populations generally very low. They occurred to be 0.05 to 0.2 per cell in 0.6-2.0% MH and from 0.05 to 0.15 per cell in 0.08-0.2% 6-BAP concentrations respectively (Tables-5 & 6).

(iii) UNSYNCHRONIZED MOVEMENT :

Unsynchronized movement of chromosomes was absent in control as well as upto 0.1% MH and 0.06 % 6-BAP. However, their frequency increased from 0.05 to 0.15 per cell in 0.2-2.0% MH and from 0.1 to 0.15 per cell in 0.08-0.2% 6-BAP respectively (Tables-5 & 6).

(iv) UNEQUAL SEPARATION AND STICKINESS :

Unequal separation of chromosomes and stickiness could not be observed in control to 0.1% MH and upto 0.08% 6-BAP. However, in still higher doses they increased from 0.05 and 0.1 per cell to 0.15 per cell in 0.2-2.0% MH and from 0.5 and 0.1 per cell to 0.1 and 0.2% in 0.2 to 2.0% MH and in 0.1 to 2.0% 6-BAP concentrations respectively (Tables-5 & 6).

(IV) ABNORMALITIES AT TELOPHASE I :

The chromosomal abnormalities in control and lower doses were generally absent at telophase I. However the micronuclei occurred from 0.10-0.20 per cell in 0.02-2% MH and in 0.01-0.2% 6-BAP respectively (Tables-5 & 6). The second meiotic stages did not show these abnormalities. However, insignificant appearance of micro nuclei, laggards and fragments could be seen rarely.

Table-1 Variations in germination and morphological characters at seedling stage induced by maleic hydrazide (MH).

Concentrations of maleic hydrazide (MH)	**Germination (%) (30 days old)**	**Seed germination injury (%)**	**Frequency of variations at seedling stage (30 days old)**	**Average height at seedling stage (cms)**	**Height injury at seedling stage (%)**
Control	55.33	0.00	0.00	17.18	0.00
0.05 %	54.66	1.20	3.00	16.14	6.05
0.1 %	49.33	10.84	10.00	15.23	11.35
0.2 %	46.00	16.86	10.00	14.52	15.48
0.4 %	45.33	18.07	13.00	14.42	16.06
0.6 %	45.33	18.07	16.00	14.35	16.47
0.8 %	44.66	19.27	16.00	11.51	33.00
1.0 %	44.66	19.27	16.00	11.14	35.15
1.5 %	44.66	19.27	17.00	10.97	36.14
2.0 %	42.66	22.89	18.00	10.95	36.26

Table -2 Variations in germination and morphological characters at mature stage induced by maleic hydrazide (MH).

Concentrations at maleic hydrazide (MH)	Frequency of variations at mature stage	Average height at mature stage	Height injury % at mature stage	Average no. of branches per plant	Days to maturity	Pollen fertility	Yield per plant	
							No. of seeds	Wt./100 seeds
Control	0.00	77.96	0.00	0.85	112	98.50	975	5.75
0.05 %	10	75.17	3.35	0.82	112	94.57	812	5.67
0.1 %	20	71.07	8.83	0.75	112	93.48	695	5.20
0.2 %	20	70.85	9.06	0.70	102	92.39	705	4.58
0.4 %	30	70.47	9.58	0.67	100	89.78	684	4.95
0.6 %	40	70.39	9.71	0.67	105	88.00	666	3.15
0.8 %	40	69.96	10.26	0.67	94	88.74	641	2.80
1.0 %	50	69.62	10.32	0.65	96	87.78	638	2.57
1.5 %	60	68.51	10.69	0.61	90	81.68	579	2.70
2.0 %	60	67.91	12.12	0.57	89	80.64	542	2.69

Table-3 : Variations in germination and morphological characters at seedling stage induced by 6-Benzyl amino purine (6-BAP).

Concentrations of 6-BAP (%)	Germination (%) (30 days old)	Seed germination injury (%)	Frequency of variations at seedling stage (30 days old)	Average height at seedling stage (cms)	Height injury at seedling stage (%)
Control	55.33	0.00	0.00	17.18	0.00
0.005 %	50.66	1.29	15.00	14.44	6.55
0.01 %	50.00	2.59	15.00	14.39	7.27
0.02 %	48.66	5.19	15.00	14.28	15.19
0.04 %	48.00	6.49	16.00	13.06	17.53
0.06 %	47.33	7.79	17.00	12.79	22.72
0.08 %	47.33	7.79	17.00	11.90	23.50
0.1 %	46.66	9.09	18.00	11.67	24.22
0.15 %	46.66	12.98	19.00	11.46	25.58
0.2 %	41.33	19.48	19.00	10.61	31.10

Table-4 : Variations in germination and morphological characters at mature stage induced by 6-Benzyl amino purine (6-BAP).

Concentrations of 6-BAP (%)	Frequency of variations at mature stage	Average height at mature stage	Height injury at mature stage	Average no. of branches per plant	Days to maturity	Pollen fertility	Yield perplant	
							No. of seeds	Wt./100 seeds
Control	0.00	77.96	0.00	0.85	112	98.50	975	5.75
0.005 %	20	72.22	1.08	0.67	102	98.17	910	5.68
0.01 %	20	72.07	1.20	0.64	100	93.47	870	5.12
0.02 %	30	71.98	1.77	0.64	99	92.83	710	4.68
0.04 %	40	71.56	1.81	0.64	99	89.74	712	4.59
0.06 %	50	70.63	3.06	0.62	94	88.79	860	3.79
0.08 %	50	70.36	3.43	0.57	90	79.78	869	3.88
0.1 %	60	66.95	4.09	0.55	89	80.57	695	3.51
0.15 %	60	69.88	5.27	0.54	80	80.65	668	2.89
0.2 %	70	69.02	8.11	0.50	77	81.89	650	2.17

Table -5 : Chromosomal studies in *Helianthus annuus* L. var. 'MSFH-8' treated with maleic hydrazide (M_1 Generation).

Concentrations (%) Meiotic Stages	Control (MH)			0.05% (MH)			0.1% (MH)			0.2% (MH)			0.4% (MH)		
	M	SD	CV	M	SD	CV	M	SD	CV	M	SD	CV	M	SD	CV
***Prophase I* (Diakinesis)**															
Freq. of chiasmata/cell	23.55	2.65	11.25	22.40	3.18	14.19	22.20	3.29	14.81	22.15	2.59	11.69	21.70	2.32	10.69
" " chiasmata/bivalent	1.38	0.15	10.86	1.31	0.18	13.74	1.30	0.19	14.61	1.29	0.15	11.62	1.27	0.13	10.23
Freq. of Multivalents/cell	-	-	-	0.10	0.43	430.0	0.20	0.87	435.00	0.10	0.43	430.00	0.10	0.43	430.00
" " Univalents/cell	3.90	2.40	61.53	5.70	2.21	38.77	5.20	2.63	50.57	4.50	2.67	59.33	5.90	2.32	39.32
" " Stickiness/cell	0.05	0.21	420.00	0.10	0.30	300.00	0.15	0.35	233.33	0.15	0.35	233.33	0.10	0.30	300.00
Metaphase I															
Freq. of chiasmata /cell	22.73	2.83	12.45	21.15	3.81	18.01	20.6	3.81	18.49	20.40	2.87	14.06	20.20	4.64	22.97
" "chiasmata /bivalent	1.33	0.16	12.03	1.24	0.22	17.74	1.20	0.22	18.33	1.20	0.16	13.33	1.18	0.27	22.88
Freq. of Multivalents/cell	-	-	-	0.10	0.43	430.0	0.25	1.08	432.00	-	-	-	0.10	0.43	430.00
" " Univalents/cell	1.55	2.87	185.16	6.90	4.12	59.71	6.80	3.86	56.76	6.60	2.97	45.00	7.90	3.71	46.96
" " Stickiness/cell	-	-	-	-	-	-	0.10	0.30	300.00	-	-	-	0.20	0.40	200.00
" " Precocious sep. of chr./cell	-	-	-	-	-	-	-	-	-	0.10	0.43	430.00	0.10	0.30	300.00
Anaphase I															
Freq. of Bridges/cell	-	-	-	0.05	0.21	420.00	0.10	0.30	300.00	0.10	0.43	430.00	0.05	0.21	420.00
" " Laggards/cell	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
" " Unsynchronized mov. of chr./cell	-	-	-	-	-	-	-	-	-	0.05	0.21	420.00	0.05	0.21	420.00
" " unequal sep. of chr./cell	-	-	-	-	-	-	-	-	-	0.05	0.21	420.00	0.10	0.43	430.00
" " Stickiness/cell	-	-	-	-	-	-	0.05	0.21	420.00	0.10	0.30	300.00	0.05	0.21	420.00
Telophase I															
Freq. of micronuclei/cell	-	-	-	-	-	-	-	-	-	0.10	0.30	300.00	0.05	0.21	420.00

M = Mean, SD = Standard Deviation, CV = Coefficient of variations

Contd...

Table-5 : Chromosomal studies in *Helianthus annuus* L. var. 'MSFH-8' treated with maleic hydrazide (M_1 Generation).

Concentrations (%) Meiotic Stages	0.6% (MH)			0.8% (MH)			1.0% (MH)			1.5% (MH)			2.0% (MH)		
	M	SD	CV	M	SD	CV	M	SD	CV	M	SD	CV	M	SD	CV
***Prophase I* (Diakinesis)**															
Freq. of chiasmata/cell	21.65	4.39	20.27	21.20	2.13	10.04	21.25	2.29	10.77	21.75	3.94	18.11	19.29	4.25	22.03
" " chiasmata/bivalent	1.26	0.25	19.84	1.24	0.12	9.67	1.24	0.13	10.48	1.27	0.23	18.11	1.12	0.25	22.32
Freq. of Multivalents/cell	-	-	-	-	-	-	0.10	0.30	300.00	0.20	0.87	435.00	0.20	0.87	435.00
" " Univalents/cell	5.10	3.60	70.58	6.90	1.94	28.11	5.70	3.42	60.00	4.90	2.48	50.60	5.90	2.32	39.32
" " Stickiness/cell	-	-	-	0.05	0.21	420.00	-	-	-	0.10	0.30	300.00	0.20	0.40	200.00
Metaphase I															
Freq. of chiasmata /cell	20.40	2.92	14.31	20.30	3.42	16.84	19.85	4.62	23.27	19.00	3.93	20.68	19.30	3.43	17.77
" "chiasmata /bivalent	1.19	0.17	14.28	1.14	0.28	24.56	1.16	0.27	23.27	1.11	0.23	20.72	1.13	0.20	17.69
Freq. of Multivalents/cell	0.20	0.87	435.00	0.30	0.95	316.66	0.20	0.87	435.00	0.30	0.95	316.66	0.20	0.87	435.00
" " Univalents/cell	7.80	3.84	49.23	8.20	3.45	42.07	7.60	3.97	52.23	9.47	3.76	39.70	13.10	20.16	153.89
" " Stickiness/cell	0.10	0.30	300.00	0.20	0.40	200.00	0.10	0.30	300.00	0.20	0.40	200.00	0.20	0.40	200.00
" " Precocious sep. of chr./cell	0.10	0.30	300.00	0.10	0.43	430.00	0.15	0.47	313.33	0.15	0.47	313.33	0.45	1.20	266.66
Anaphase I															
Freq. of Bridges/cell	0.10	0.30	300.00	0.10	0.30	300.00	0.15	0.47	313.33	0.15	0.47	313.33	0.15	0.47	313.33
" " Laggards/cell	0.05	0.21	420.00	0.15	0.47	313.33	0.15	0.47	313.33	0.20	0.50	250.00	0.20	0.50	250.00
" " Unsynchronized mov. of chr./cell	0.15	0.35	233.33	0.10	0.43	430.00	0.15	0.35	233.33	0.10	0.43	430.00	0.15	0.35	233.33
" " unequal sep. of chr./cell	0.10	0.30	300.00	0.10	0.43	430.00	0.10	0.30	300.00	0.15	0.47	313.33	0.15	0.47	313.33
" " Stickiness/cell	0.10	0.43	430.00	0.15	0.47	313.33	0.10	0.30	300.00	0.10	0.43	430.00	0.15	0.47	313.33
Telophase I															
Freq. of micronuclei/cell	-	-	-	0.15	0.47	313.33	0.10	0.43	430.00	0.20	0.67	335.00	0.20	0.67	335.00

M = Mean, SD = Standard Deviation, CV = Coefficient of variations

Table 6 : Chromosomal studies in *Helianthus annuus* L. var. 'MSFH-8' treated with 6-Benzylamino purine (M_1 Generation).

Concentrations (%) Meiotic Stages	Control (6-BAP)			0.005% (6-BAP)			0.01% (6-BAP)			0.02% (6-BAP)			0.04% (6-BAP)		
	M	SD	CV	M	SD	CV	M	SD	CV	M	SD	CV	M	SD	CV
***Prophase I* (Diakinesis)**															
Freq. of chiasmata/cell	23.55	2.65	11.25	21.30	3.70	17.37	21.60	4.02	18.61	20.60	6.06	29.41	23.00	7.25	31.52
" " chiasmata/bivalent	1.38	0.15	10.86	1.24	0.21	16.93	1.26	0.23	18.25	1.29	0.24	18.60	1.34	0.42	31.34
Freq. of Multivalents/cell	-	-	-	0.10	0.30	300.00	0.20	0.87	435.00	0.10	0.43	430.00	0.10	0.30	300.00
" " Univalents/cell	3.90	2.40	61.53	6.35	1.85	29.13	6.60	2.13	32.27	5.95	2.10	35.29	6.10	2.86	46.88
" " Stickiness/cell	0.05	0.21	42.00	-	-	-	0.10	0.30	300.00	0.10	0.30	300.00	0.10	0.43	430.00
Metaphase I															
Freq. of chiasmata /cell	22.73	2.83	12.45	21.65	2.83	13.07	19.10	3.92	20.52	19.50	4.59	23.53	19.95	5.02	25.16
" "chiasmata /bivalent	1.33	0.16	12.03	1.27	0.16	12.59	1.11	0.22	19.81	1.14	0.32	28.07	1.17	0.17	14.52
Freq. of Multivalents/cell	-	-	-	-	-	-	-	-	-	0.10	0.43	430.00	0.20	0.87	435.00
" " Univalents/cell	1.55	2.87	185.16	5.55	2.87	51.71	7.35	2.95	40.13	7.29	2.54	34.84	7.20	1.64	22.78
" " Stickiness/cell	-	-	-	-	-	-	0.05	0.21	420.00	0.10	0.30	300.00	0.05	0.21	420.0
" " Precocious sep. of chr./cell	-	-	-	-	-	-	0.10	0.30	300.00	0.10	0.43	430.00	0.15	0.47	313.33
Anaphase I															
Freq. of Bridges/cell	-	-	-	-	-	-	0.05	0.21	420.00	0.05	0.21	420.00	0.10	0.30	300.00
" " Laggards/cell	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
" " Unsynchronized mov. of chr./cell	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
" " unequal sep. of chr./cell	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
" " Stickiness/cell	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Telophase I															
Freq. of micronuclei/cell	-	-	-	-	-	-	0.10	0.43	430.00	0.20	0.67	335.00	0.10	0.30	300.00

M = Mean, SD = Standard Deviation, CV = Coefficient of variations

Table 6 : Chromosomal studies in *Helianthus annuus* L. var. 'MSFH-8' treated with 6-Benzylamino purine (M_1 Generation) *Contd...*

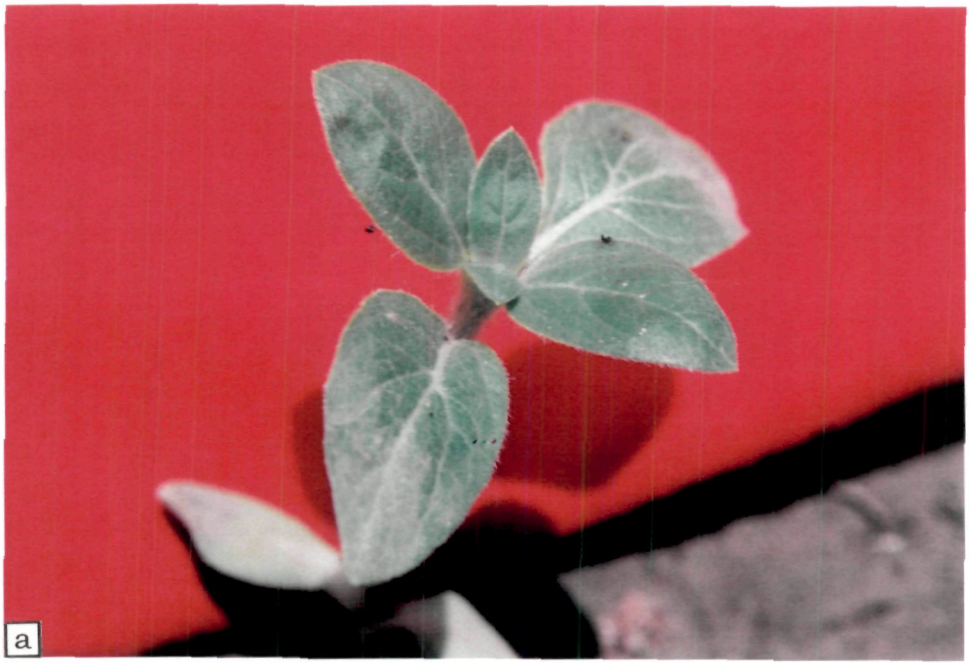
Concentrations (%) Meiotic Stages	0.06% (6-BAP)			0.08% (6-BAP)			0.1% (6-BAP)			0.15% (6-BAP)			0.2% (6-BAP)		
	M	SD	CV	M	SD	CV	M	SD	CV	M	SD	CV	M	SD	CV
***Prophase I* (Diakinesis)**															
Freq. Of chiasmata/cell	22.20	5.61	25.27	22.00	4.04	18.36	19.95	4.00	20.05	23.00	6.04	26.26	19.85	3.65	18.38
" " chiasmata/bivalent	1.30	0.33	25.38	1.28	0.23	17.96	1.16	0.23	19.82	1.34	0.35	26.11	1.16	0.21	18.10
Freq. of Multivalent/cell	0.10	0.43	430.00	0.20	0.87	435.00	0.50	1.50	300.00	0.10	0.43	430.00	0.20	0.87	435.00
" " Univalent/cell	6.10	2.50	40.98	5.80	2.20	37.93	7.05	2.22	31.48	5.50	3.02	54.90	7.70	3.59	0.46
" " Stickiness/cell	0.05	0.21	420.00	0.15	0.47	313.33	0.05	0.21	420.00	0.10	0.43	430.00	0.15	0.47	313.33
Metaphase I															
Freq. of chiasmata /cell	19.20	3.96	20.62	20.00	5.05	25.25	20.40	2.87	14.06	19.45	5.26	27.04	17.40	4.82	27.70
" "chiasmata /bivalent	1.12	0.23	20.53	1.17	0.29	24.78	1.20	0.16	13.33	1.14	0.20	17.54	1.01	0.28	27.72
Freq. of Multivalent/cell	0.10	0.43	430.00	0.50	1.50	300.00	0.10	0.43	430.00	0.20	0.87	435.00	0.50	1.50	300.00
" " Univalent/cell	7.40	1.98	26.75	7.50	1.91	25.46	6.60	2.97	45.00	6.15	2.08	33.82	6.25	2.34	37.44
" " Stickiness/cell	0.15	0.47	313.33	0.15	0.47	313.33	0.10	0.30	300.00	0.10	0.30	300.00	0.15	0.47	300.00
" " Precocious sep. of chr./cell	0.10	0.43	430.00	0.45	1.20	266.66	0.15	0.47	313.33	0.20	0.67	335.00	0.45	1.20	266.66
Anaphase I															
Freq. of Bridges/cell	-	-	-	-	-	-	0.10	0.43	430.00	0.15	0.35	233.33	0.15	0.35	233.33
" " Laggards/cell	-	-	-	0.05	0.21	420.00	0.05	0.21	420.00	0.10	0.43	430.00	0.15	0.47	313.33
" " Unsynchronized mov. of chr./cell	-	-	-	0.10	0.43	430.00	0.15	0.47	313.33	0.10	0.30	300.00	0.15	0.47	313.33
" " unequal sep. of chr./cell	-	-	-	-	-	-	0.05	0.21	420.00	0.05	0.21	420.00	0.10	0.43	430.00
" " Stickiness/cell	-	-	-	-	-	-	0.05	0.21	420.00	0.01	0.3	300.00	0.20	0.67	335.00
Telophase I															
Freq. of micronuclei/cell	0.10	0.43	430.00	0.10	0.43	430.00	0.20	0.67	335.00	0.10	0.30	300.00	0.20	0.67	335.00

M = Mean, SD = Standard Deviation, CV = Coefficient of variations

PLATE 1 : EXPLANATION OF FIGURES

Fig. (a) : **Control Seedlings:**
Normal growth, leaves cordate, hairy entire, acute, opposite at early stage, may be alternate at older and mature stages.

Fig. (b) : **Control Seedlings:**
Normal growth, erect, opposite, cordate, entire acute leaves.



a

Control

b

Control

Figure Plate -1 (a, b)

Morphological variations in *Helianthus annuus L.* treated with maleic hydrazide (M.H.)

PLATE 2 : EXPLANATION OF FIGURES

Fig. (a) : C ordate oblong leaves, thick leathery and hairy, entire, acute, one leaf a cuminate, opposite and one leaf deformed shrunken.

Fig. (b) : The plant bearing oval, oblong, broader and acute as well obtuse leaves. Average length of leaves reduced.

0.05% (M.H)

0.1% (M.H)

Figure Plate -2 (a, b)

Morphological variations in *Helianthus annuus L.* treated with maleic hydrazide (M.H.)

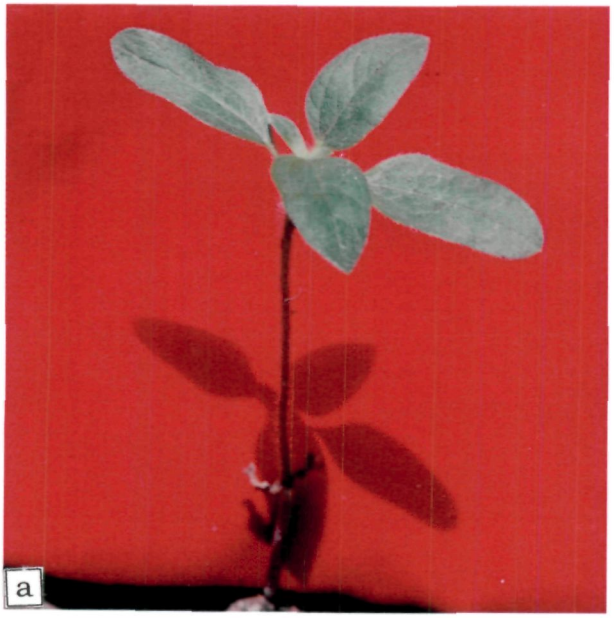
PLATE 3 : EXPLANATION OF FIGURES

Fig.(a) : Plant bearing two types of leaves: Cordate, entire, acute and oblong leaves.

Fig.(b) : Cotyledonary leaves oval cum rectangular, vegetative leaves diverted to one side, oblong with obtuse apices, condensed nodes, Rough surface, darkgreen colour. Planterect showing stunted growth.

Fig.(c) : Whorl like arrangement (condensed at the apex.) wavy and dissected margin, obovate as well as rhomboid, hairy surface as well as entire margin.

Fig.(d) : Hairy stem, obovate & ovate leaves, deshaping in one leaf (0.4% MH).



0.1% B (M.H)

0.2% (M.H)

0.4% (M.H)

0.4% (M.H)

Figure Plate -3 (a, b, c & d)

Morphological variations in *Helianthus annuus L.* treated with maleic hydrazide (M.H.)

PLATE 4 : EXPLANATION OF FIGURES

Fig. (a) : Plant showing slow growth (reduced height), oval as well as oblong leaves showing entire margine and acute apices.

Fig. (b) : Cordate as well as oval-oblong leaves showing acute as well as obtuse apices.

Fig. (c) : Plant showing stunted growth, reduced size of leaves with deshaping. One leaf rudimentary.

Fig. (d) : Plant showing stunted growth, oval as well as ovate leaves.

0.6% (M.H)

0.8% (M.H)

1.0% (M.H)

1.0% (M.H)

Figure Plate -4 (a, b, c & d)

Morphological variations in *Helianthus annuus L.* treated with maleic hydrazide (M.H.)

PLATE 5 : EXPLANATION OF FIGURES

Fig.(a) : Plant showing growth, leaves ovate oblong, entire acute.

Fig.(b) : Broader leaves, deep notching forming two overlapping lobes (emarginated), one leaf cordate, three leaves i.e. leaves of different shape and size.

Fig.(c) : Thick but semierect stem, bearing normal but unegnal Ctyledonary leaves, All vegetative leaves rud imentary condensed at the apex.

Fig.(d) : Seedling showing stunted growth (smallest height) leaves normal but smaller.

1.5% (M.H)

1.5% (M.H)

2.0% (M.H)

2.0% (M.H)

Figure Plate -5 (a, b, c & d)

Morphological variations in *Helianthus annuus L.* treated with 6-Benzyl amino purine (6-BAP)

PLATE 6 : EXPLANATION OF FIGURES

Fig. (a) : Leaves showing notching and varigate d chlorophyll deficiency. Some leaves are round (orbicular) with notching. All other leaves generally smaller in size and ovate.

Fig.(b) : Cotyledonary leaves showing deshaped structure, retuse, ovates oblong.

0.005% 6-BAP

0.01% 6-BAP

Figure Plate -6

Morphological variations in *Helianthus annuus L.* treated with 6-Benzyl amino purine (6-BAP)

PLATE 7 : EXPLANATION OF FIGURES

Fig.(a) : Some seedlings are showing deshaped leaves. Decreased number of germination.

Fig.(b) : Ovate and oblong leaves. Some seedlings have deshaped and chlorophyll deficient leaves.

a

0.02% 6-BAP

b

0.04% 6-BAP

Figure Plate -7

Morphological variations in *Helianthus annuus L.* treated with 6-Benzyl amino purine (6-BAP)

PLATE 8 : EXPLANATION OF FIGURES

Fig. (a) : Cordate, deltoid, oblong and chlorophyll deficient leaves in affected seedlings one seedling shows notching stage.

Fig. (b) : Leaves are cordate, ovate & oblong, Rhomboid, slightly notched, chlorophyll deficiency and D-shaped structure in cotyledonary leaves.

0.06% 6-BAP

0.08% 6-BAP

Figure Plate -8

Morphological variations in *Helianthus annuus L.* treated with 6-Benzyl amino purine (6-BAP)

PLATE 9 : EXPLANATION OF FIGURES

Fig. (a) : The leaves oval, broader, obtuse as well as acute apexes.

Fig.(b) : Seedling showing general variations in shape and size of leaves such as notching, cordate, oblong, ovate etc. Some leaves show chlorophyll deficiency.

0.1% 6-BAP

0.15% 6-BAP

Figure Plate -9

PLATE 1 : CHROMOSOMAL STUDIES AT DIAKINESIS

Fig. (a) : **Diakinesis (Control) :**
14 Rings and 6 Univalents.

Fig. (b) : **Diakinesis :**
11 Rings and 6 Rods (MH – 04%)

Fig. (c) : **Diakinesis :**
9 Rings, 5 Rods and 6 Univalents (MH – 1.0%)

Fig. (d) : **Diakinesis :**
9 Rings, 6 Rods and 4 Univalents (3 biavlents still at diplotene stage). (6-BAP-0.02%)

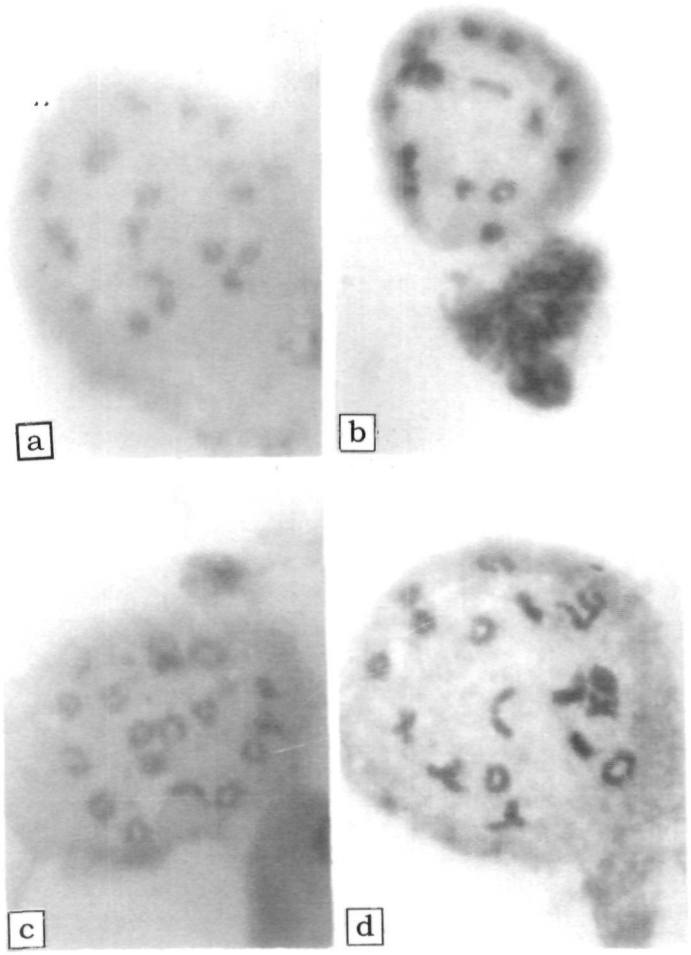


Figure Plate -1

PLATE 2 : CHROMOSOMAL STUDIES AT METAPHASE

Fig. (a) : **Metaphase I :**
Showing stray chromosomes (all univalents), (MH – 0.1%)

Fig. (b) : **Metaphase I :**
2 Univalents (one on either side). Astray chromosomes, all Univalents (MH – 0.5%)

Fig. (c) : **Metaphase I :**
Precocious separation of chromosomes. One univalent on either side (6-BAP – 0.04%)

Figure Plate -2

PLATE 3 : CHROMOSOMAL STUDIES AT ANAPHASE

Fig. (a) : **Anaphase I :**
Normal, equal division of 17+17 chromosomes (univalents).

Fig. (b) : **Anaphase I :**
Unequal division (17+16+1 laggard) (MH – 0.8%)

Fig. (c) : **Anaphase I :**
Broken chromatin bridge (6-BAP-0.01%)

Fig. (d) : **Anaphase I :**
Stickiness, unequal division at two poles. (6-BAP-0.1%)

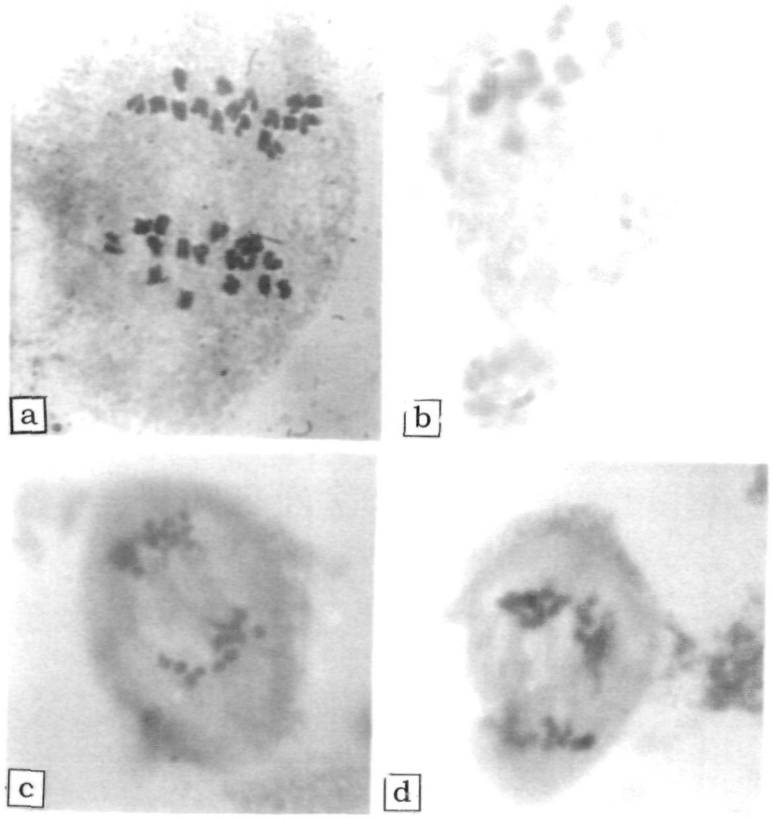


Figure Plate -3

PLATE 4 : CHROMOSOMAL STUDIES AT ANAPHASE

Fig. (a) : **Anaphase I :**
2 laggards but with equal division of chromosomes. (16+16+2 laggards) (MH – 0.2%)

Fig. (b) : **Anaphase I :**
Stickiness resulting in stretching of chromatin material (MH-0.6%).

Fig. (c) : **Anaphase I :**
Laggards at late stage (6-BAP – 0.04)

Fig. (d) : **Anaphase I :**
2 bridges, 2 laggards and stickiness. (6-BAP – 0.08%)

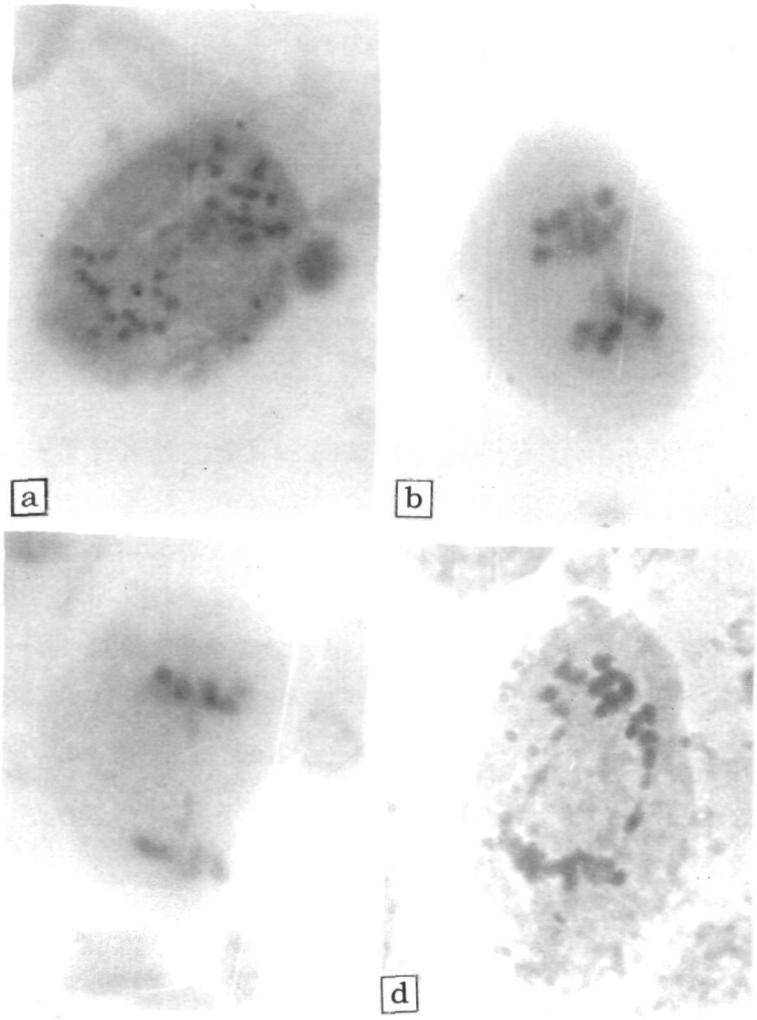


Figure Plate -4

PLATE 5 : CHROMOSOMAL STUDIES AT METAPHASE AND TELOPHASE

Fig. (a) : **Telophase I :**
Trace of broken bridge (broken due to separation of chromosomes). (MH. – 0.8%)

Fig. (b) : **Metaphase II :**
Normal (MH – 1.5%)

Fig. (c) : **Telophase II :**
5 groups : chromosomes arranged in 5groups. (one group of chromosomes divided in two unequal groups). (6-BAP – 0.15%)

Fig. (d) : **Telophase II :**
Tripolar orientation of chromosomes. (6-BAP – 0.2%)

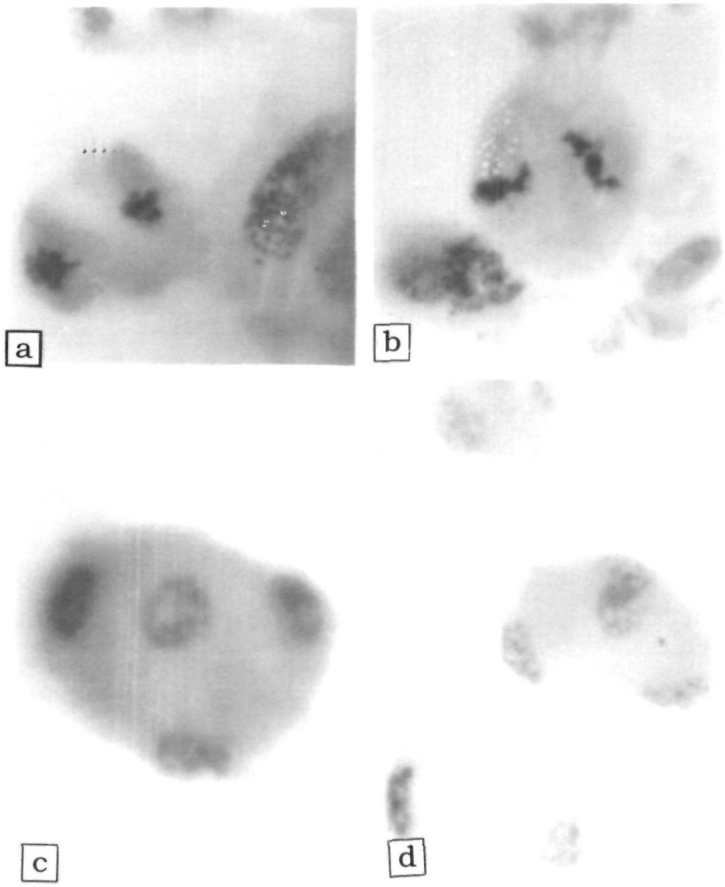


Figure Plate -5

Discussion

CHAPTER – 5

DISCUSSION

The present discussion is mainly confined to the effect of maleic hydrazide and 6- benzyl amino purine, on seed germination, seedling morphology, plant morphology, cytology, yield and other characters of *Helianthus annuus L.*variety "MSFH-8".The probable reasons and explanations regarding the mutagenic effects have been discussed.

The breeding potential of a crop is to exploit the existing variability through selection or created variability, but the breeding method is time and labour oriented.On the other hand, mutation breeding technique is quicker and the best method to enlarge the genetically conditioned variability of a species within a short time and has played a significant role in the development of many crop varieties.

SEED GERMINATION

The test of germination is an important parameter to estimate the induced effects of the mutagen on metabolism resumed after a period of dormancy. Generally the germination decreased with the increasing concentrations of MH and 6-BAP in the present investigation. The reduced germination due to inhibitory effect, as observed in *Helianthus annuus* L. has been reported in pulses (Sahai,

1974) and tomato (Khamankar,1974). Similar results have also been reported by many workers (Basu and Basu,1968; Rajput 1974; Patel and Shah,1974; Siddiqui *et al.*,1979,1980 ; Chandra and Tiwari , 1978; Krishna *et al.*,1984; Sloan and Camper , 1986) .

Several workers have attempted to explain the causes responsible for inhibition of germination in both mutagens. It may be due to damage to the enzyme system involved in repair mechanism or due to the production of toxic substances in the cell. Griffiths and Johnson (1962) and Srivastava (1979) considered it to be due to weakening and disturbances of growth processes.

Several workers have made attempts to explain the causes responsible for inhibition of seed germination.The destruction of growth regulators (Sideris *et al.*,1969) and metabolic disturbances during germination (Ananthaswamy *et al.*,1971) may be some of the reasons .In *Helianthus annuus* the decreased germination was reported by Corbineau *et al.* (1988) by the treat ment of methyl jasmonate .

Gay *et al.* (1991) reported inhibition in germination at high temperature in *Helianthus annuus* L., whearas increase in germination has been reported by the increase of temperature from 8^0C to 34^0C (White and Montes,1993). Baxter *et al.* (1994) observed that smoke and smoke extracts stimulate seed germination of the fire

climax grass .Griffiths and Johnson (1962) and Srivastava (1979) considered that reduction in germination percentage was due to weakening and disturbances of growth processes regulated in early elimination of seedlings . The inhibition of growth regulators and metabolic disturbances during germination may also be one of the reasons (Sideris *et at.*,1971). Seed germination, average height, branches etc. decreased with the increasing concentrations of MH and 6-BAP treat ments in the present investigation. However, the extent of decrease differed in both mutagens. Number of changes have been indicated after mutagenic treat ments (Grover and Tejpaul , 1979 ; Mahapatra,1983 ; Adamska *et al.*, 1995 ; Pandey *et al.*, 1996 ; Kumar and Mani , 1997 ; Arumugam *et al.*, 1997 ; Khan *et al.*, 1998 ; Anis *et al.*,1999). Most of these workers have observed a dose dependent reduction in germination. Several workers attempted to explain the causes responsible of inhibition for germination. Kalia (1984) reported that the inhibition of germination in chemical treatments may be due to damage to the enzyme system involved in repair mechanism or due to the production of toxic substances in the cell.

SEEDLING GROWTH

The average seedling height exhibited decreasing trend as compared to control. However, some individuals exhibited increased height over control. As observed in *Helianthus annuus* at present, the similar effects of reduction in growth has been reported by many

workers such as, Krishna *et al.* (1984) in *Chloris gayana,* Temple (1990) in *Lycopersicon esculentum* and Murray and Wilson (1991) in *Medicago truncatula.* Qussible *et al.* (1992) reported that in wheat the sub surface compection also reduced the seedling growth. Corradi *et al.* (1993) observed that the chromium reduced the seedling growth in *Salvia scalaria,* while pre-sowing treatment of seeds with concentrated sulphuric acid produced better results for seedling growth (Bardwaj and Chakraborty, 1994).

Various workers have attempted to explain the phenomenon of reduced seedling growth by mutagenic treatments. The most probable causes are suggested to be uneven damage to the meristematic cells as a consequence of genetic injuries (Gray and Scholes,1951), structural changes in the constitution of chromosomes (Thoday,1951),chromosomal damages or inhibition of cell division (Sparrow *et al.*, 1958), the chromosomal damage and in part due to physiological disturbances which affected the height (Russel and Martin 1962) .Sparrow and Sparrow (1965) concluded that the growth inhibition arises from the interference with the cell elongation . Inhibition of impaired mitosis could also be the reason for reduced growth (Mergan and Johnson, 1964) .Sparrow *et al.* (1958) reported that reduced stem elongation is due to reduced nutrition contents. The mechanism of assimilation may also be important factor.

ABNORMALITIES IN LEAF MORPHOLOGY:

At seedling stage the abnormalities such as shape and size of cotyledonary and vegetative leaves were commonly observed and their frequency increased with increasing concentrations of both mutagens. Similar result as observed in *Helianthus annuus L.* are common effects of mutagens in different plants reported by many other workers , such as , Basu (1962) in *Corchorus* species , Nayer and George (1969) in *Brassica juncea* , Bajaj *et al.* (1970) in *Phaseolus vulgaris* , Silvy (1984) in barley , Krishna *et al.* (1984) in *Chloris gayana,* Sloan and Camper (1986) in cucumber. Cheng and Gao (1988) in barley , Zeerak (1990) in brinjal , Murray and Wilson (1991) in *Medicago truncatula* and Corradi *et al.* (1993) in *Salvia sclarea.*

According to Devreux and Mugnozza (1964) the disturbances in metabolic activities due to mutagenic treatements may be one of the important factors responsible for leaf anamolies in plants. Hagen and Gunckel (1958) found in general that where leaf abnormalities occurred there was a concomitant increase in the free amino acid contents in these leaves. Similar factor may be responsible for leaf variations in MH and 6-BAP also.

ABNORMALITIES IN MATURE PLANTS:

The effect of MH and 6-BAP on height of mature plants is quite pronounced as compared to control.The lower doses due to being less

toxic produced variations but the injury was negligible, while the higher doses retarded the growth. However, the effect of mutagen on the height of mature plants is rather linear and dose dependent. Moreover the frequency of variations was higher in higher doses providing more chances for selection. Similar adverse effect on plant growth has also been observed by gamma irradiation in *Citrullus lanatus* (Katiyar and Roy1972, 1973). *Sesamum* (Nath 1974), Linseed (Seetharam 1976; Nath 1978), *Brassica campestris* (Kumar *et al.*, 1977) *Coriandrum sativum* (Sinha and Sinha 1977), *Solanum khasianum* (Chauhan 1978), barley (Singh *et al.*,1980).Sparrow *et al.* (1958) mentioned that although reduced stem elongation is usually ascribed to reduced auxin and nutritional levels , the mechanism of assimilation may also be important factor . On the whole it appears to be complex biological phenomenon operating at cellular levels as well as sub-cellular levels.

POLLEN FERTILITY:

The pollen fertility showed variations in treated populations and overall it decreased with the increasing concentrations of MH and 6-BAP.Sinha and Godward (1969, 1972 a. b) described the translocations to be responsible for decreased pollen fertility. Rana and Swaminathan (1964) and Ramanna (1974) reported that any deviation in karyokinesis and cytokinesis could produce nonviable microspores.

The effect of both mutagens on pollen fertility has also been reported in *Arachis hypogea* and *Plantago ovata* (Bora *et al.*, 1961) , *Carthamus tinctorius* (Chauhan 1969) , *Cyamopsis tetragonoloba* (Rai 1971), *Sesamum indicum* and *Martynia dyandra* (Nath 1974), *Luffa cylindrica* and *Luffa acutangula* (Katiyar and Roy 1974, 1975), *Brassica campestris* (Kumar *et al.*, 1977), *Chloris gayana* Kunth (Krishna *et al.*,1984), *Arachis hypogea l.* (Dutta *et al.*,1986). Moreover pollen germination and pollen tube growth was found to be affected by a gamma rays (Cung and Roudot 1991). Thus various chromosomal abnormalities like translocation, anaphase bridges, laggards, etc. induced by mutagens lead to decreased pollen fertility in *Helianthus annuus l.* also.

In the present study varying degrees of pollen sterility were induced in both the mutagenic treat ments. Generally the magnitude of sterility was considerably higher and increased with the increase in mutagenic concentrations. Similar reports have been made earlier by Ganguli and Bhaduri (1980) Prasad and Das (1980 b) , Bhadra (1982), Nadarajan *et al.* (1983) and Kumar and Dubey 1994, 1998a,b) .

The high sterility observed in the treated populations may be attributed to the vast array of meiotic aberrations that were induced by the MH and 6-BAP leading to aberrant pollen mother cells and ultimately in the inactivation of pollen grains. This is in agreement

with many workers (Rana and Swaminathan , 1964 ; Sinha and Godward , 1972a, b ; Ramanna 1974) .

MEIOTIC ABNORMALITIES:

Mutations can be beneficially utilized for tailoring better varieties of crop plants. But in general, ionizing radiations and chemical mutagens cause a wide range of chromosomal alterations resulting into abnormal behaviour during meiosis, leading to various degrees of meiotic irregularities like lowered chiasmata frequency, multivalents, univalents, disturbed bivalent associations, stickiness, laggards, fragments, precocious separation of chromosomes, unequal division, bridges with or without fragments, which generally increased with the increasing concentrations of maleic hydrazide and 6-benzyl amino purine. Moreover, the frequency of these abnormalities was higher in meiosis I than in meiosis II. Further, the cytological abnormalities during meiosis have also been regarded as one of the dependable parameters for estimating mutagenic sensitivity of a species (Damyanthi and Reddy, 2000).

Cytogenetic studies are also important for obtaining informations regarding the role and effect of various mutagens (MH and 6-BAP) and elucidating the response of various genotypes to a particular mutagen (Reddy and Annadurai, 1992). In this context, cytological investigations appear to be rewarding as they deal with the

primary genetic material, the chromosome and more appropriately the DNA which, controls the phenotypes. The best approach would be to consider the chromosomes as the source of genetic informations necessary for the development of phenotypes (Katiyar 1978).

At the same time recovery mechanism can start operating in the elimination of lethal and sub- lethals through natural selection right from the inhibition of germination following mutagenic treatment (Sinha and Godward, 1969). Yet some aberrations persist and effect the viability of gametes and subsequently the fertility of plant. Different types of meiotic abnormalities observed in the present investigation have also been reported by different workers in different plant materials after treatment with physical and chemical mutagens, viz. Singh and Roy (1971) in *Trigonella foenum – graecum,,* Tarar and Dyansagar (1980) in *Turnera ulmifolia* , Ahmad and Godward (1981) in Chick pea, Kumar and Dubey (1998 c) in *Lathyrus sativus* , Mishra and Raghuwanshi (1988) in *Trigonella foenum- graecum* , Zeerak (1992 a) in *Lycopersicon,* Mitra and Bhowmik (1996) and in *Nigella sativa* , Anis and Wani (1997) in *Trigonella foenum – graecum* , Anis *et al.* (1999) and Dhamyanthi and Reddy (2000) in *Capsicum annuum and* Verma *et al.* (1999) in *Lens culinaris.* Most of these workers have obtained a dose dependent increase in meiotic aberrations. Varietal sensitivity to mutagenic treatements was also reported by some workers .

MULTIVALENTS :

The formation of multivalents by maleic hydrazide and 6- Benzyl aminopurine as described here has also been reported by many workers. Heiner *et al.* (1960), Konzak *et al.* (1962) and Sree Ramulu (1971) working with gamma rays and DES reported that the chemical mutagens produce chromosomal aberrations less frequently than radiations. The quadrivalents (Translocation rings) as observed in *Helianthus annuus* were also observed in *Pennisetum typhoides* (Burton and Powell, 1966; Srinivasachar and Mohandas, 1971), *Lycopersicon esculentum* (Zeerak 1992) and *Cicer arietinum* (Saeed 1993) by physical and chemical mutagenic treatments .

STICKINESS :

Stickiness among chromosomes was the most common abnormality observed in the present investigation. Chromosomes were clumped into one, two or many groups due to stickiness at metaphase causing difficulty in normal disjunction of chromosomes. These results are in agreement with Sinha and Godward (1972b); Katiyar (1978), Tarar and Dnyansagar (1980 a) and Mitra and Bhowmik (1996), who also reported stickiness and grouping of different bivalents as the most common abnormality. This could be due to a partial dissociation of the nucleoproteins and alterations in their pattern of organization (Anis and Sharma, 1997) or due to the

depolymerisation of nucleic acid caused by mutagenic treatment (Tarar and Dnyansagar, 1980). It has also been reported by Ragab and Raheem (1989) in *Zea mays*, Zeerak (1992) in *Lycopersicon esculentum* and Saeed (1993) in *Cicer arietinum* by the treatment of some insect growth regulator, EMS and gamma rays respectively. Darlington and La cour (1945) suggested that there was a reduction of correctly polymerized nucleic acid on the chromosomes producing characteristic errors of spiralization which combined with superimposed excess of non – polymerized nucleic acid to cause surface stickiness .

According to Rangaswami (1935) and Magoon *et al.* (1958) the lagging nature of chromosomes seems to be due to some trouble in terminalization of chiasmata resulting from change in homology of the paired chromosomes .

PRECOCIOUS MOVEMENT OF CHROMOSOMES :

The precocious separations of chromosomes at metaphase I as observed in the present material has been attributed to crossing over between relatively inverted segments (Mc Clintock , 1938) or the reunion of chromatids during meiotic prophase (Rees and Thompson 1955 , Lewis and John 1966 , Newmann 1966) .

Precocious separation was also observed in *Arachis hypogea* (Patil and Bora , 1961) raised from x-ray irradiated seeds . Bose and

Saha (1970) concluded that the univalents separating precociously seemed to be as a result of desynapsis . According to Roy *et al.* (1971) precocious separation of bivalents at metaphase I in *Cucumis sativus* was attributed to the failure of chiasma formation in pairs . In *Turnera ulmifolia* the precocious separation was also observed by gamma rays and EMS treatments (Tarar and Dnyansagar 1980 b).

UNIVALENTS :

The univalents at diakinesis and metaphase I were later found to become laggards at anaphase and telophase stages . These seem to arise from partial or lack of homologous chromosomes pairing or due to cryptic structural changes in some of the chromosomes which strict pairing. Rao and Laxmi (1980) attributed the univalent formation to the partial and complete lack of homologous chromosome pairing, Further, the disturbances in the pairing was ascribed to the presence of chromosome breakage in the PMCs of plants raised from treated seeds. Some of the univalents disjuncted earlier and presumably this happened due to genic differences in synchronized terminalization of chiasmata. Mitra and Bhowmik (1996) reported that non- pairing and early separation of chromosomes at meiosis may result in the formation of univalents. Zeerak (1992) concluded that mutagen-induced structural changes in chromosomes and gene mutations might be responsible for the failure of pairing among homologous chromosomes and hence the presence of univalents .

LAGGARDS :

The laggards observed during the present investigation may be due to delayed terminalization of chiasmata, stickiness of chromosome ends or because of failure of chromosomal movement (Jayabalan and Rao, 1987, Soheir *et al.*, 1989). Schulz (1980) concluded that lagging chromosomes and their presence as univalent may result in aneuploidy. The occurrence of laggards has also been observed by Rangaswami (1935) in *Pennisetum typhoides* , Ammini (1968) in *Rhoeo discolor*, Bose and Maiti (1971) in *Lycopersicon esculentum* Mill., Roy *et al.*, (1971) in *Cucumis sativus*, Sree Ramulu (1971) in Sorghum, Ragab and Raheem (1989) in *Zea mays, Lycopersicon esculentum, Raphanus sativus, Brassica campestris, Lagenaria vulgaris, Solanum surattense, Datura alba* and *Argemone maxicana*, Al- Safadis and Simon (1990) in *Daucus carota L.*, Zeerak (1992) in *Lycopersicon esculentum L.* and Khare (1994) in *Adiantum capillus* .

According to Tarar and Dnyansagar (1980) unsynchronized bivalents or laggards might be due to discrepancies in spindle formation .

BRIDGES :

Bridges without fragments at anaphase and telophase stages were frequently observed in the present investigation . Bridges often break as the chromosomes move further apart in the late anaphase .

The presence of chromosome bridges without fragments may be due to restitution or the fragments getting entangled or attached with normal chromatids of chromosomes (Tarar and Dnyansagar, 1980). Jones (1968)described the contrasting modes of origin of bridges in simple terms "one depends on the crossing over between relatively inverted segments, the other depends on inverted crossing over between non-oriented segments." It is also possible that bridges may result due to stickiness of chromosome ends (Carlson 1954, Sawamura 1965, Sudhakaran 1971).Thomas (1961) observed that in some cells the interstitial chiasmata in the translocated chromosomes fails to terminalize completely and during anaphase this results in bridge .

Bridge formation was also observed by Ragab and Raheem(1989), Kumar *et al.* (1989), Al-Safadis and Simon (1990), Zeerak (1992) and Saeed (1993) in different plants .

The cells showing unequal division may arise when one chromosome of a quadrivalent goes to one pole and remaining three to the other .It is also possible that a bivalent may fail to disjoin and move as a whole to one of the poles . Unequal distribution was also observed in *Pennisetum typhoides* (Krishnaswamy and Ayyanger 1941) by x-rays, tomato (Bose and Saha 1970) by DES and x-rays, *Rhoeo discolor* (Ammini, 1968) by maleic hydrazide , *zea mays* (Ragab and Raheem, 1989) by insect growth regulator, *Cicer arietinum* (Saeed,

1993) by gamma-rays and *Adiantum capillus* (Khare, 1994) by gamma rays.

Movements of bivalents towards poles at anaphase due to non-disjunction of homologous chromosomes at metaphase as observed during the present study was due to stickiness of chromosomes and could result in unequal distribution of chromosomes in the daughter nuclei.

Abnormalities such as lagging chromosomes and unequal separation of chromosomes would lead to the production of aneuploid gametes and thus the aneuploid plants in the next generation .Such plants (aneuploids) are of immense importance in fundamental as well as applied research in crop improvement. Among different stages of meiosis, the frequency of meiotic aberrations was maximum at metaphase stage in the present study.

CONCLUSION

It has been concluded that the cyto-morphological variations observed in the present experiment may be due to the physiological, biochemical, metabolic and chromosomal/genic disturbances induced by the action of MH and 6-BAP, along with their interactions with the environment.

MH showed linear concentration effect relationship on different aspects of *H. annuus* L., such as seed germination, growth and yield etc., while 6-BAP on the other hand, showed enhancing effect on these aspects in lower concentrations. Moreover, in higher concentrations the effect of 6-BAP was similar to those in MH. Morphological variations were higher in MH and 6-BAP treatments in both varieties in M_1 generation. Chromosomal abnormalities were also concentration dependent and more or less responsible for morphological variations. The mutations were more or less similar in both mutagens with the differences in their frequencies only.

It is apparent that MH and 6-BAP have potentiality to induce variability in *H. annuus* L. and therefore, could be used to improve the crop both in terms of quantitative and qualitative characters.

Summary

CHAPTER – 6

SUMMARY

The effect of mutagens maleic hydrazide (MH) and 6-benzyl amino purine (6-BAP) on seed germination,seedling and plant growth, meiotic chromosomes,pollen fertility,yield and life span of *Helianthus annuus* L. variety "MSFH-8" has been studied in M_1 generation.The findings are summarized below.

1. Lower doses of MH and 6-BAP caused no mild effect on seed germination but in higher concentrarions delayed and decreased germination was observed.

2. Lower doses of MH and 6-BAP exhibited stimulatory effect on seedling growth while in higher doses the height of the seedlings decreased.

3. The cotyledonary leaves of treated population exhibited abnormalities like decreased angles; fusion at base upto half of the length or even upto 3/4 length forming a heart shaped structure, poorly developed, notched, string like, rudimentary leaves in higher doses. Most of the abnormalities were common in all the doses, with the difference in their frequencies.

4. First pair of vegetative leaves also exhibited morphological variations like poor development, deshaping, whorl formation,

lobe formation, notching, chang in serration and apex, fusion, rudimentary leaves.

5. Older and mature plants seedlings exhibited morphological variations like varied leaf sizes, weak stem, healthy seedlings with branching in higher doses of mutagens.Besides this plyllotaxy was also affected in treated populations in M_1 generation. Variations like increased notching and dissection of lobes formation was observed.

6. Different types of meiotic abnormalities were also observed in treated populations in different frequencies (bivalents, multivalents, fragments, formation of bridges, laggards, stickiness, unsynchronized division etc.

7. Yield showed an decreasing trend with increasing concentrations. Some individual plants shwoed important changes such as ....

8. Mathematical and statistical analysis of the data recorded in M_1 generation particularly seed germination, pollen fertility, yield, morphological variations, chromosomal configurations etc. has been carried out to establish the statistical relationship between concentrations of mutagous (MH and 6-BAP) and different cytomorphological abnormalities of *Helianthus annuus* L.

9. Moreover similar types of works have been reviewed and possible reasons behind these variations have been discussed.

FUTURE PLAN OF WORK

The work of mutagenesis on *Helianthus annuus L.* variety "MSFH-8" have been started by the treatment of chemical mutagens (maleic hydrazide and 6-benzyl amino purine). Important variants have been obtained in the treated populations and isolated in M_1 generation. Their morphological as well as cytological characters have been studied. Further plan of work is summarized as under :-

1) Attempts will be made to obtain improved mutants/ variants through the induction of mutations. The variants obtained in M_1 have been selected separately after selfing the affected plants. The seeds of such plants have been sown in M2. In which the true mutants (recessive mutants) will be detected out and maintained separately on the basis of change in habit, seedling and plant morphology , branching, flowering, fruiting, yield seed morphology, cytogenetic studies etc. The mutants will be protected from contamination by selfing processes.

2) The seeds obtained in M_2 from these selected mutants as well as from general treated populations will be sown in M_3 generation to find out segregation, if any, and to estabtish the true mutants. In M_3 generation also the inflorescences will be selfed to maintain homozygosity.

3) The variety "MSFH-8" will be assessed for comparative efficiency and effectiveness of mutagens on the basis of morphological and cytological parameters mentioned above.

4) Production of polyploids (autotetraploids) will be attempted in order to obtain better quality, bigger seeds and higher yield by treatment of aqueous solution of colchicine.

5) Cytogenetic studies of control, randomly selected plants of treated populations,selected mutants and colchiploids will be carried out in detail in M_1, M_2 and M_3 generations. Specially the numerical and morphological changes, pairing behaviour of chromosomes like bivalents, univalent, multivalents, chiasma frequency, bridges, laggards etc. will be studied. A relationship between morphological variations of the plants and structural and morphological variations of chromosomes will be worked out.

6) The decreased or increased oil contents, oil constituents, protein and other chemical contents will be estimated in mutants as compared to control.

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